NOTES ON THE ISOLATION OF THE TISSUE ELEMENTS.

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In the present period when the technique of section cutting has become so perfect that many of the cells of the body may be cut into several pieces, there is some danger of losing sight of the actual conformation of the cells as wholes. Certainly, as teachers of histology, it is desirable for us to show our students as many of the cells or tissue elements as possible so that they may realise that the teacher is discussing, real, tangible entities when he speaks of epithelial cells, muscle cells or brain cells, and the like. Furthermore, the student should gain an allround conception, so to speak, and this notion of the tissue elements is gained by the student only when he can see all around the structures; this feat is easily accomplished in the isolated cells by causing them to roll over with a little pressure on the cover-glass.

In dissociating, the aim is to separate the tissue elements from one another, the cells and all their minute processes being preserved in their true form. In order to do this the cell-cement, or intercellular substance, must be dissolved or softened. The perfect dissociator then, must harden the tissue elements and soften the substance which holds them together. Many excellent dissociators have been described. None will serve equally well for all tissues, and there may be a "best dissociator" for each animal; it seemed worth while, however, to present a note upon the results of an extended series of experiments to discover if possible the general and underlying principles.

The general principles seem to be these: *Any agent which acts as a good hardening and fixing medium for a tissue will also serve for a dissociating substance if sufficiently diluted and allowed to act only a short time.* So far as experiments have gone it was found that if the fixer suitable for a tissue were diluted ten times and allowed to act from two hours to two days, good results were obtained in isolating. It was further found that if the diluting substance used were normal salt solution (water 1000 cc., common salt 6 grams,) the results were, perhaps, more satisfactory. This use of normal salt solution was suggested from the fact that it tends to leave the tissues without change, and the diffusion currents are not so severe as when water alone is used for dilution.

For the epithelia of mucous and serous surfaces nothing was found so satisfactory for all animals as formaldehyde in normal salt solution. The strength used was 2 cc. in a liter of normal salt solution. For many epithelia the isolation may be considered sufficient in one to two hours; good preparations from the same may be got after a day or two. This dissociator is excellent for obtaining the ciliated cells of the brain ventricles. And in experimenting with it for that purpose it was found that the nerve cells of the cerebral cortex were most satisfactorily isolated also. For one who has only seen nerve cells in sections it would be a revelation to see the processes as shown in such isolation preparations, and then if the cells be made to roll over, it will be seen that the cells have processes projecting from every side.

No method of studying the isolated elements has been so successful as scraping off a small mass and mounting on a slide in the dissociating medium, and then for the more complete separation the cover-glass is gently hammered over the mass of cells. The mechanical jarring separates the cells without tearing them, and often two or more cells are just sufficiently separated to show their mutual relation. It is sometimes advantageous to add a little eosin solution to the

mass of cells before mounting or after, but as the mounting medium, if the dissociator is used, is of such different refractive index, all the structural details come out without stains; and it is worth while to let the student see that histological structure can be seen under the microscope without gorgeous stains. He will then know, which I fear is not always the case now, that the cells are not red and purple in the living body.

If in examining preparations mounted only in the dissociator one should meet with something that he was extremely desirous of preserving, the slide may be laid flat and a drop of glycerin put at the edge of the cover. It will partly diffuse and also as the dissociator evaporates it will run in by capillarity and in a few days the preparation will be mounted in glycerin. It may then be sealed with shellac or other cement and will last a reasonable length of time, that is, till one naturally gets a better preparation to take its place.

If one wishes to have the cells stained for the permanent preparations, instead of using glycerin alone, as just described, the following mixture will be found excellent: Glycerin, 85 c.c.; alum carmine, $7\frac{1}{2}$ c.c.; eosin, $\frac{1}{2}$ per cent. aqueous solution, $7\frac{1}{2}$ c.c. This may be put at the edge of the cover as for the glycerin, or preferably it should be mixed with the cells before putting on the cover-glass. The alum carmine stains the nuclei and the eosin the cell body.